Thyrocalcitonin: Evidence for Release in a Spontaneous Hypocalcemic Disorder

Abstract. *Thyrocalcitonin content of thyroid gland extracts from normal postparturient cows was 3.9 times greater than in cows with postparturient paresis. The parafollicular cells in diseased cows were less numerous and appeared to have discharged their secretory products. An abrupt release of thyrocalcitonin near parturition may be related to the development of the hypocalcemia and hypophosphatemia in this disorder.*

Thyrocalcitonin, a hypocalcemic and hypophosphatemic polypeptide, has been demonstrated in extracts of thyroid glands from many different mammalian species including cattle and man (*1*). Numerous investigators have since postulated the physiologic importance of thyrocalcitonin in calcium homeostasis. There are indications that thyrocalcitonin, produced by the parafollicular (thyroid C-) cells, may play a significant role in the pathogenesis of certain metabolic diseases, namely, pseudohypoparathyroidism and nontoxic goiter of man (*2*) and osteopetrosis in mice (*3*). It has also been advocated for the treatment of idiopathic hypercalcemia of infants (*4*). A spontaneous metabolic disease of cattle (parturient paresis) was selected as an experimental model to study the role of thyrocalcitonin in the development of hypocalcemia and hypophosphatemia at parturition. We tested the hypothesis that an abrupt release of a significant amount of the stored thyrocalcitonin at a critical time near parturition could result in a precipitous decrease in the serum calcium and phosphorus. We found support for this hypothesis by correlating ultrastructural evaluation of parafollicular cells with biologic assay for thyrocalcitonin in the thyroid glands of normal and affected cows.

Three normal postparturient cows and three cows with postparturient paresis were used in our study. The thyroid and parathyroid glands were removed immediately after euthanasia, and 0.5-mm cubes were fixed in osmium tetroxide for electron microscopy. The remaining thyroid tissue was frozen (−90°C) for biologic assay. Extracts were prepared from the thyroid glands of each cow by a two-step procedure consisting of homogenization and ultracentrifugation at 4°C. The thyroid glands were thawed rapidly and dissected free of connective tissue, internal parathyroid glands, and fat. Thyroid tissue was homogenized in 0.1*N* HCl (10 ml/gm) with Potter-Elvehjem grinders. The homogenate was centrifuged at 20,000*g* for 25 minutes in a preparative ultracentrifuge. The supernatant was centrifuged again at 100,000*g* for 24 hours. Total protein determinations were performed on the final supernatant (*5*).

Thyrocalcitonin biologic assay was performed by the method of Hirsch *et al.* (*1*). Intact male Holtzman rats (49 to 51 days old) were maintained on a calcium-deficient diet (*6*) for 4 days and were fasted 14 hours prior to assay. Seven rats were randomly assigned to each of three doses (1, 2, and 4 mg of protein) for each thyroid extract. Control rats and those injected with HCl were included in each assay. Thyroid gland extracts from normal postparturient controls and from cows with postparturient paresis were injected into parallel groups of assay rats. All rats were anesthetized with ether and exsanguinated from the abdominal aorta 60 minutes after injection. Serum calcium concentrations in the test rats were determined by atomic absorption spectrophotometry, and a log dose–response curve was plotted as described (*1*). The unit of thyrocalcitonin is an arbitrary one and is equal to the quantity (in milligrams) of protein in the thyroid extract required to produce a decrease of 1 mg/100 ml in the serum calcium of assay rats 60 minutes after subcutaneous injection. The mean concentration of thyrocalcitonin in the thyroid glands (units per gram) was determined from the results of the biologic assay and protein analysis. The potency ratio (normal : diseased) of thyrocalcitonin activity was calculated (*7*).

Table 1. The mean thyrocalcitonin (TCT) concentration for normal cows and for cows with postparturient paresis was 63.9 and 15.6 units per gram, respectively. Cows with postparturient paresis had a severe hypocalcemia and hypophosphatemia. The concentration of serum calcium and phosphorus in cow 6 was increased in view of intravenous treatment with calcium and phosphorus 24 hours prior to collection of the thyroid glands.

Cow	TCT (units per gram of thyroid)	Serum values (mg/100 ml)	
		Ca	P_i
Postparturient controls			
1	43.2	8.2	4.2
2	89.1	10.0	4.5
3	59.4	9.2	5.6
Postparturient paresis			
4	13.4	3.8	2.1
5	13.6	3.6	1.5
6	19.8	6.8	3.6

There was a distinct difference in the thyrocalcitonin concentration (units per milligram of extract protein) between the two groups of cows. The mean quantity of extract protein required to produce a 1-unit response in assay rats was 0.4 mg for normal cows and 2.0 mg for cows with postparturient paresis. The potency ratio revealed that the thyrocalcitonin content was 3.9 times greater in normal postparturient cows than in cows with postparturient paresis. In the control cows there appeared to be a relation between the gland concentration of thyrocalcitonin and the amount of serum calcium (Table 1). The cow with the greatest concentration of thyrocalcitonin had the highest serum calcium value, and the cow with the least concentration had the lowest serum calcium. Cow 6 received an intravenous injection of calcium (8.4 g) and phosphorus (4.8 g) 24 hours prior to euthanasia and had recovered clinically from postparturient paresis. The thyrocalcitonin concentration in the thyroid gland was greater than in untreated cows 4 and 5.

The parafollicular (thyroid C-) cells of normal postparturient cows were larger and less electron-dense than follicular cells, and were found singly in an intrafollicular location or in small groups between thyroid follicles (Fig. 1). They contained a large Golgi apparatus associated with numerous prosecretory granules, aggregated rough endoplasmic reticulum, scattered mitochondria, and a high concentration of spherical secretory granules and vesicles limited by a single membrane. The ultrastructural appearance was indicative of high secretory activity.

Parafollicular cells in the thyroid glands of cows with postparturient paresis were less numerous and appeared to have discharged most of their secretory products (Fig. 2). Secretory granules and vesicles were infrequent, and the Golgi apparatus was small. The cells contained a few ribosomes, sparse

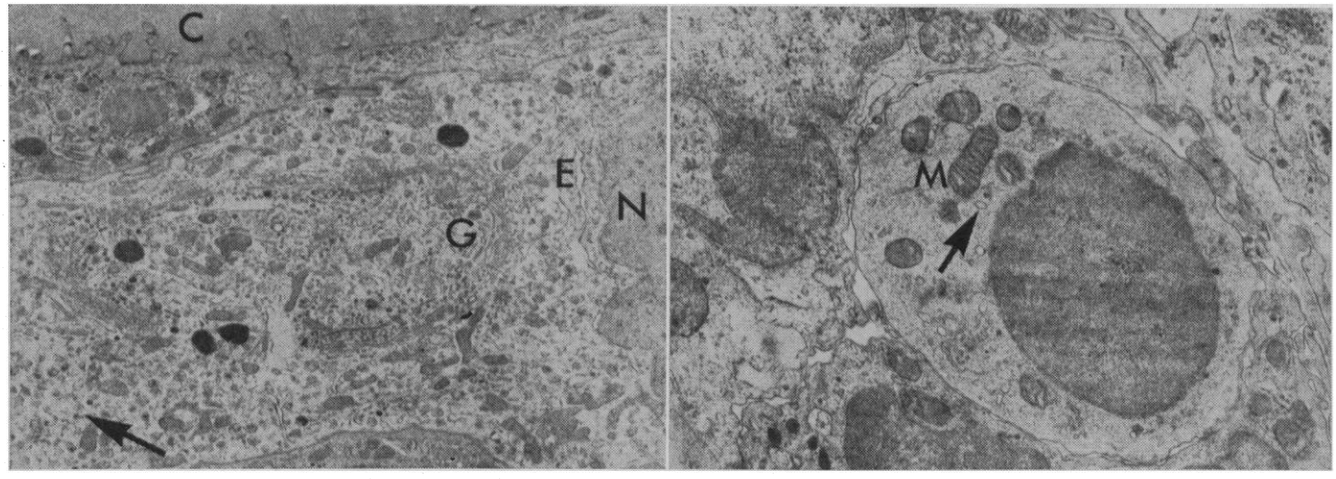

Fig. 1 (left). Parafollicular cell in the thyroid gland of a normal postparturient cow. *N*, part of nucleus. Numerous secretory granules (arrow), a large Golgi apparatus (*G*), and endoplasmic reticulum (*E*) are present in the cytoplasm. Microvilli of follicular cells project into thyroid colloid (*C*). × 10,000. Fig. 2 (right). Parafollicular cell from a cow with postparturient paresis and hypocalcemia. Secretory granules (arrow) are infrequent. The cytoplasm contains mitochondria (*M*) and ribosomes. × 12,500.

endoplasmic reticulum, and scattered mitochondria. These cells resembled the discharged or degranulated parafollicular cells reported by Pearse (*8*) and Matsuzawa and Kurosumi (*9*) to be in thyroid glands after hypercalcemic perfusion.

The parathyroid glands of cows with postparturient paresis had definite ultrastructural evidence of increased synthesis and secretion of parathyroid hormone compared to normal postparturient cows. Chief cells were either depleted of mature secretory granules or contained a few granules situated peripherally near the plasma membrane. The Golgi complex was enlarged and associated with numerous prosecretory granules and vacuoles. Ribosomes and membranes of the endoplasmic reticulum were aggregated, and the plasma membranes of apposing chief cells were intricately interdigitated. This morphologic evidence of parathyroid hyperactivity correlates well with the reported elevation in plasma parathyroid hormone concentration (determined by radioimmunoassay) in cows with parturient paresis as compared to control cows (*10*). The administration of exogenous parathyroid hormone is of no benefit in the treatment of bovine parturient paresis (*11, 12*). Therefore, a deficiency of parathyroid gland function does not appear to be the basic defect in this condition.

Other investigations offer support for the hypothesis that the discharged parafollicular cells and diminished thyrocalcitonin content in the thyroid glands of cows with parturient paresis are related to the cause, rather than an effect, of the hypocalcemia. With a hypocalcemia produced by other causes, the thyrocalcitonin content is increased, and parafollicular cells are packed with secretory granules (*13, 14*). In rats made hypocalcemic by parathyroidectomy, parafollicular cells were reported to be more numerous and packed with secretory granules, compared with those cells in normal and hyperparathyroid rats (*13*). The thyrocalcitonin content in the thyroid glands of the hypocalcemic rats was 2.7 times greater than that of intact controls (*14*). Gittes *et al.* (*14*) concluded that the synthesized hormone is stored in excess of normal during hypocalcemia in the rat when thyrocalcitonin release is at a minimum. The stimulus for the apparent sudden release of thyrocalcitonin in cows that developed postparturient paresis is not known. Hypercalcemia has been reported as the stimulus for thyrocalcitonin release in other species (*15*). Parturition in the cow is usually associated with amounts of serum calcium and phosphorus in the lower part of the physiologic range. The increased parathyroid hormone secretion that accompanies the onset of parturition in cows (*11*) could possibly result in a transient hypercalcemia of sufficient magnitude to stimulate the release of thyrocalcitonin. An inhibition by thyrocalcitonin (*16*) of bone resorption stimulated by parathyroid hormone could have resulted in the precipitous decrease of serum calcium and phosphorus in cows with postparturient paresis. Care *et al.* (*15*) reported that the hypocalcemic response appeared to be an all-or-none type of effect when a certain degree of hypercalcemia was attained.

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